1: Biochem Pharmacol. 2002 Aug 15;64(4):669-75.

Effects of antifolates on the binding of 5-fluoro-2'-deoxyuridine monophosphate to thymidylate synthase.

van der Wilt CL, Smid K, Peters GJ.

Department of Medical Oncology, VU University, Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands.

Folate based inhibitors of thymidylate synthase (TS) might facilitate binding of 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) to TS similar to the natural reduced folate 5,10-methylenetetrahydrofolate (CH(2)-H(4)-folate). We studied the lipophilic, non-polyglutamatable antifolates Nolatrexed (NTX) and AG331 and antifolates, that can have a polyglutamate side chain like the natural folate CH(2)-H(4)-folate; GW1843U89, Raltitrexed (RTX) and Multi-targetted antifolate (MTA) and pentaglutamates (RTX-Glu(5) and MTA-Glu(5)). The capacity of these compounds to facilitate the binding of [(3)H]FdUMP to Lactobacillus casei TS and an ammoniumsulphate precipitate of human TS was investigated. Only NTX, RTX-Glu(5) and MTA-Glu(5) facilitated FdUMP binding to L. casei TS and their dissociation constant K(d) (0.2-0.7 microM) was low compared to CH(2)-H(4)-folate (2.0 microM). The small lipophilic molecule NTX was favorable to the larger AG331. Polyglutamylation, as indicated by the difference in effect of RTX vs. RTX-Glu(5) and MTA vs. MTA-Glu(5), seems to be important for a classical antifolate to facilitate binding of FdUMP to bacterial TS. Effects of antifolates on FdUMP binding to human TS were different. At a low concentration (0.05 microM) NTX, RTX-Glu(5) and MTA-Glu(5) facilitated 3-5 times higher binding of [(3)H]FdUMP to TS than CH(2)-H(4)-folate. At higher concentrations (0.3-5 microM) of NTX, RTX-Glu(5) and MTA-Glu(5) the FdUMP binding decreased. The complex remained stable in the absence of (anti)folate for at least 24hr. The K(d) values of the antifolates for human TS varied from 19 to 387 nM, while the K(d) of CH(2)-H(4)-folate for human TS was 351 nM. The Hill coefficients, which indicated the type of cooperativity of the antifolates in the binding of FdUMP to TS were positive (0.58-0.99) at low concentrations (<0.3 microM) and negative (-0.35 to -0.81) at concentrations >0.3 microM except for GW1843U89, which only showed negative cooperativity (-1.70).

It was shown with [(14)C]NTX that when the binding of FdUMP decreased at high NTX concentrations, the binding of NTX to TS still increased. This also held for the natural substrate dUMP. The negative cooperativity of the antifolates was clearly concentration dependent. The difference between human and L. casei TS in the FdUMP binding assays with antifolates can possibly be explained by interaction of the two subunits of human TS, which was absent in L. casei TS. The binding of antifolates to one of the two subunits induced a conformational change of the other subunit. This change no longer allowed the binding of FdUMP or dUMP at the active site. In conclusion this study showed that antifolates enhanced the binding of FdUMP to TS, especially at low antifolate concentrations, that are also clinically achievable, e.g. in human plasma.

PMID: 12167486 [PubMed - indexed for MEDLINE]
